AWARD NUMBER: W81XWH-15-1-0084

TITLE: Cancer Risks Associated With Inherited Mutations in Ovarian Cancer Susceptibility Genes Beyond BRCA1 and BRCA2

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REPORT DATE: May 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
May 2017	Annual	4/15/2016 – 4/14/2017

4. TITLE AND SUBTITLE

Cancer Risks Associated With Inherited Mutations in Ovarian Cancer Susceptibility Genes Beyond BRCA1 and BRCA2

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0084

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Ovarian, peritoneal and fallopian tube carcinomas (OC) are the most deadly of the gynecological cancers. Our data indicate that at least 20% of unselected OC is hereditary and that 20-25% of inherited mutations occur in genes other than *BRCA1* and *BRCA2*. The large fraction of OC associated with inherited mutations in a variety of genes provides an important opportunity to reduce OC mortality. Maximizing the benefit from OC risk assessment and prevention requires an improved understanding of the penetrance of OC genes beyond *BRCA1/2*. Furthermore, minimal data exist regarding the hereditary component of OC, including *BRCA1/2*, in non-white populations. The objective of this study is to define the genetic causes of hereditary OC in African Americans (AA) as well as the spectrum of cancers, the age of onset, and the relative risk associated with mutations in non-*BRCA1/2* genes. In year 2, we have enrolled an additional 98 high risk probands and 8 AA probands with OC for BROCA sequencing of 45 known or candidate OC genes. We continue to enroll probands and their relatives to better understand the genetic contribution to ovarian cancer and will focus on exome sequencing 30 families in year 3.

15. SUBJECT TERMS
Ovarian cancer, drug resistance, rucaparib, phase 2, DNA repair, homologous recombination, nonhomologous end-joining (NHEJ), poly(ADP-ribose)polymerase, BRCA1, BRCA2, PARP1

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	UU	9	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION

Ovarian, peritoneal and fallopian tube carcinomas (OC) are the most deadly of the gynecological cancers and can be considered together as one entity. While women with early stage OC have an excellent chance of cure, attempts to improve early detection have been largely ineffective. In contrast to surveillance, surgical prophylaxis with risk-reducing salpingo-oophorectomy (RRSO) reduces OC mortality in high risk women. Inherited mutations in *BRCA1* and *BRCA2* (*BRCA1/2*) account for about 15% of OC. Inherited loss of function mutations in other related genes account for another 5-6% of cases, but less is understood about the OC risk associated with mutations in these genes. Furthermore, there are other OC genes that have not yet been discovered. Our hypothesis is that rare, inherited, damaging mutations in genes other than *BRCA1/2* confer a relatively high cancer risk that would warrant age appropriate surgical prophylaxis. A better understanding of the etiologic contribution from, and penetrance of, genes other than *BRCA1/2* to hereditary OC is needed to guide clinical decision-making and to optimize recommendations for OC prevention. Our overall goal is to refine the understanding of inherited OC susceptibility, emphasizing genetic variation in diverse racial populations and genes other than *BRCA1* and *BRCA2*. We will achieve these objectives through two specific aims:
Aim 1: Identify rare variants in OC susceptibility genes other than *BRCA1/2* in women with ovarian, fallopian tube or peritoneal carcinoma who have an increased likelihood of genetic risk.
Aim 2: Identify the genetic contribution of many genes to OC susceptibility among African American women with ovarian, fallopian tube or peritoneal carcinoma.

2. KEY WORDS

Ovarian cancer, BRCA1, BRCA2, cancer susceptibility, RAD51C, RAD51D, PALB2, BRIP1, BARD1, African-American, familial, hereditary

3. ACCOMPLISHMENTS

The first goal was to enroll and BROCA sequence 200 high risk probands in years 1 and 2 and that has been accomplished (N=234), which includes 98 patients since our last progress report. Our BROCA targeted sequencing assay includes 11 known OC genes: *BRCA1, BRCA2 (FANCD1), BARD1, BRIP1 (FANCJ), RAD51C (FANCO), RAD51D, PALB2 (FANCO),MSH2, MLH1, MSH6, PMS2,* 9 other known breast cancer genes: *ATM, CHEK2, FAM175A (abraxas), FAMCM, NBN, PTEN, RECQL, TP53* and *XRCC2*) and 25 other candidate genes in the Fanconi anemia-BRCA pathway: *ATR, BABAM1, BAP1, BLM, BRCC3, BRE, CHEK1, ERCC1, ERCC4 (FANCQ), FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG (XRCCC9), FANCI, FANCL, GEN1, MRE11A, RAD50, RAD51, RBBP8 (CtIP), SLX4 (FANCP), UIMC1 (RAP80).* All damaging mutations have been confirmed with Sanger Sequencing.

Of the 98 newly enrolled high risk subject, sequencing has been completed on 64 and is in process on 34. All probands had ovarian, fallopian tube, or primary peritoneal carcinoma confirmed on a pathology report. Patients also had a first or second degree relative with ovarian carcinoma and/or had a second invasive non-skin cancer. Seven patients were enrolled with a known mutation in a gene of interest including BRIP1 (2), *PALB2* (2), *BARD1* (1), *RAD51D* (1), *RAD51C* (1). Putting together the data from years 1 and 2, of the 166 patients with a close relative with ovarian cancer, sequencing is pending on 34 (20%), 84 (51%) had no mutation identified in on ovarian cancer susceptibility gene, and damaging mutations in known or suspected ovarian cancer susceptibility genes were identified in 48 (29%) including *BRCA1 (19), BRCA2* (13), *ATM (1), BRIP1 (5), NBN (3), PALB2 (2), RAD51C (4), RAD51D (1),* and 1 patient had mutations in

both *MSH6* and *RAD51D.* Four patients had a damaging mutation in a gene not likely to be associated with ovarian cancer susceptibility including *CHEK2 (2), FANCA (1), and FANCL (1).* Of the 59 patients with a second cancer, most had an invasive breast cancer, but there was a variety of other cancers including uterine and thyroid. For these patients, damaging mutations were identified in a known or suspected breast or ovarian cancer genes in 16/59 (27%) including 9 *BRCA1*, 2 *BRCA2* and one each in *BLM, CHEK2 PALB2*, *RAD51D* and *RAD51C.* Mutations in *BLM* and *CHEK2* probably accounted for the patients' breast but not ovarian cancer diagnoses.

A second goal was to enroll and exome sequence 20 BROCA negative families in years 1 and 2. To date we have only exome sequenced 1 family and have found no clearly causative mutation. We decided to postpone the majority of the exome sequencing until 3 in order to select the most informative families of all enrolled. We plan to do all the planned exome sequencing in year 3.

A third goal was to enroll and sequence 100 African-American (AA) women with OC. Enrollment of AA women has lagged behind our goal. Currently we have enrolled 17 AA subjects, and completed sequencing on all. Of the 17 AA OC patients sequenced to date, 1 had a BRCA1 mutation and one patient had a frameshift mutation in *TP53*. That patient had a history of both colon and ovarian cancer. While we had many centers who were eager to send us AA patients at the time we proposed this study, the change in clinical testing has hampered our enrollment. Please see our plans described below to address this lack of AA patients.

Opportunities for training and professional development has the project provided?
Nothing to report

Dissemination of Results
Nothing to report

Plans during the next reporting period.
We will continue to recruit subjects and family members for both aims. We will use BROCA for targeted sequencing and select the most informative families for exome sequencing. For dead family members, we will obtain tissue from pathological archives when available with approval from next of kin to increase the number of ovarian cancer patients available in families for sequencing (particularly for exome sequencing).

4. IMPACT

Impact on the principal discipline
Nothing to report

Impact on other disciplines
Nothing to report

Impact on technology transfer
Nothing to report

Impact on society
Nothing to report

5. CHANGES/PROBLEMS

Changes in approach
Nothing to report

Problems or delays and plans to resolve them:

Despite having a number of collaborative centers who agreed to refer African American patients, AA patient accrual is lagging. Previously, many collaborators described a low rate of testing and coverage of testing in their AA patients. However, our collaborators have seen a change in clinical practice and an increased reimbursement for genetic testing for all OC patients. They now see that most of their AA patients are getting tested clinically, and there is therefore less motivation to enroll into a genetics research study.

As a backup strategy, we are now collaborating with Dr. Ernst Lengyel at the University of Chicago to access anonymized tissues from AA patients with OC. This should add 50 AA OC patients. We are also requesting anonymized DNA from AA OC patients who participated on clinical trials with the Gynecologic Oncology Group (now NRG Oncology).

Exome sequencing is behind schedule in order to best select the families to use this more expensive sequencing strategy. In year 3, we will proceed with the 30 most promising families.

Changes that had a significant impact on expenditures
Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

6. PRODUCTS

Publications, conference papers, and presentations
Nothing to report

Website(s) or other Internet site(s)
Nothing to report

Technologies or techniques
Nothing to report

Inventions, patent applications, and/or licenses
Nothing to report

Other Products
Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Elizabeth Swisher MD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-2331-0434
Nearest person month worked:	1
Contribution to Project:	Dr. Swisher is directing all aspects of the project including IRB oversight, recruitment, sequencing analyses, and data interpretation
Name:	Maria Harrell, PhD
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Dr. Harrell is overseeing all sequencing including quality control.
Funding Support:	
Name:	Ming Lee PhD
Project Role:	Bioinformaticist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Lee runs the bioinformatics pipeline for the next generation sequencing.
Name:	Kathy Agnew
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1

Contribution to Project:	Ms. Agnew manages all incoming samples, keeps the study database, communicates with referring provers and generates result letters.
Funding Support:	
Name:	Marc Radke
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Mr. Radke preps all sample, extracts DNA and creates library pretps for DNA sequencing. He performs Sanger sequencing validations.
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes. Dr. Swisher has the additional new support as follows:

OC160274 (Swisher) 7/1/2017-6/30/2020 8% FTE
Department of Defense, Ovarian Cancer Research Program Clinical Development Award
Mutator Phenotypes that Better Predict PARP Inhibitor Response in Ovarian Carcinomas
Role: Principal Investigator $600,000 total directs
The goal of this proposal is to identify mutator signatures characteristic of homologous recombination deficiency in tumors samples from patients on the PARP inhibitor trial ARIEL2, part 1 in order to develop a better clinical test for identifying patients who will respond to PARP inhibitors compared to currently available assays that depend on assessment of allelic imbalance.
Specific Aims
Aim 1: Identify mutator signatures suggestive of HRD using whole genome sequencing of pretreatment biopsies from subjects in the ARIEL2 trial of rucaparib in recurrent platinum-sensitive OC.
Aim 2: Compare clinical outcomes in ARIEL2 associated with each mutator signature alone or in combination relative to LOH profiling, including overall response rate, progression-free survival and duration of response.
Aim 3: Correlate mutator signatures with germline and somatic mutations in *BRCA* and other homologous recombination genes, *RAD51C* and *BRCA1* promoter methylation, and LOH signatures.
Contact:
Scientific Officer:
Karen Wylie, Ph.D.
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301-619-783

OC160355 (Swisher) 7/15/2017-7/14/2020 10% FTE
Department of Defense, Ovarian Cancer Research Program Investigator Initiated Research Award
Real-Time Assessment of Homologous Recombination Deficiency during Ovarian Cancer Treatment
Role: Principal Investigator
The goal of this proposal is to develop 1. A sequencing assay for somatic BRCA reversion mutations and 2. a homologous recombination assessment in cell-free plasma DNA to allow noninvasive monitoring of DNA repair capacity in cancer during the course of treatment.
Specific Aims:
Aim 1: Develop a next generation sequencing assay to identify somatic reversion mutations that correlate with platinum and PARP inhibitor resistance.
Aim 2. Develop a cfDNA assay to assess tumor homologous recombination status in real-time in women undergoing treatment for ovarian cancer.
Contact:
Scientific Officer:
Karen Wylie, Ph.D.
Department of Defense Ovarian Cancer Research Program
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What other organizations were involved as partners?

Nothing to report

Appendices

None